Cured Color Development During Frankfurter Processing

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SUMMARY

Batches of frankfurter emulsion were prepared using a typical meat formula and standard pilot-plant equipment. The processing variables investigated were: 1) emulsification in air, nitrogen, carbon dioxide, and nitric oxide in nitrogen; 2) use of sodium ascorbate, erythorbate, borohydride and dithionite, cysteine and glucono-delta-lactone; 3) processing at different temperatures; 4) vacuum mixing of emulsions; and 5) storage in permeable and impermeable films. No measureable stable cured meat pigment was produced during chopping. A lag period in color production, attributed to the presence of oxygen, was reduced by chopping under nitrogen, vacuum mixing, or the addition of ascorbate or cysteine. Temperature of cooking was critical to rate of color formation, percentage of cured meat pigment formed, and level of color maintained during storage. An interaction of effects between various types of wrapping film and cooking temperatures was attributed to residual biochemical activity in samples cooked to the lower temperatures.

INTRODUCTION

Continued improvement of some of the conditions under which meat pigments are converted to cured meat chromophores has drastically reduced the time required for the development of cured meat color in the processing of frankfurters. The principal chemical highlights of this progression have been the introduction of the use of nitrite in curing (Lewis et al., 1925; Kerr et al., 1926), the use of ascorbate to act as an oxygen scavenger and/or reductant to reduce nitrite (Komarik and Hall, 1951; Watts and Lehmann 1952a,b; Hollenback and Monahan, 1953; Brockmann and Morse, 1953); and, most recently, the introduction of glucono-delta-lactone for pH control (Sair, 1961). Harper (1960) patented the direct use of nitric oxide for producing color, but the technique is still in the process of development. Despite these developments, even faster cures, especially for automated processes, and greater

color stability, for modern packaging and retailing techniques, are still being sought by the industry. These demands can be fulfilled only with a thorough understanding of the reaction mechanism and the conditions which govern it.

Although reaction sequences for cured-meat color development have been postulated (Watts and Lehmann, 1952b; Bauernfeind et al., 1954; Solberg, 1966), supporting evidence is deficient. From a study of the kinetics of the formation of nitric oxide myoglobin from metmyoglobin, nitrite and ascorbate, Fox and Thomson (1963) determined the mechanism of the reaction. More recently, Walters and Taylor (1965) studying the reduction of nitrite to nitric oxide by pig muscle mitochondria, determined that the reaction was mediated by the cytochrome c oxidation-reduction system. However, those studies prove only the sufficiency of the systems studied, saying nothing as to whether or not these are the systems actually involved in cured-meat color production.

At this laboratory, investigations have been conducted to obtain information on the mechanisms of the color reactions through studies of isolated systems in the laboratory and of meat emulsions in the pilot plant. The present discussion deals with conditions during processing which affect the rate and extent of color production and includes the investigation of various reductants, temperature, pH, atmosphere, vacuum mixing, and storage stability.

The choice of reductants was dictated by common practice, as in the case of sodium ascorbate and erythorbate, or by experience of others indicating that the compounds react with nitrite or nitrous acid and/or the heme pigment to form reduced heme pigments and/or their nitric oxide complexes. Results of Kiese, (1944), Watts et al. (1955), Kelley and Watts, (1957), and Fox and Thomson (1963) suggested that the effect of cysteine should be investigated. The use of dithionite was suggested by

work of Brooks (1937), who found that the production of nitric oxide hemoglobin was extremely rapid in the presence of this reductant. Borohydride has been found capable of reducing methemoglobin (Asakura et al., 1964). Preliminary tests of the effect of borohydride on the nitrite-heme pigment reaction in this laboratory indicated that it was effective above pH 6 over a narrow range of pH, and that its reaction with the heme pigment produced a variety of unidentifiable compounds at the usual pH of meat emulsions. The effect of the reductant was investigated nevertheless, to determine the effectiveness of an additive capable of producing a very strong reducing atmosphere. The use of glucono-delta-lactone (GDL) as a pH-controlling agent was suggested by a report of Sair (1965).

MATERIALS, METHODS, AND EQUIPMENT

Emulsion formulas. Fresh beef and pork were trimmed to desired fat levels, respectively 10 and 20%. The trimmed meat and fresh pork fat were weighed into portions approximating the size needed to prepare batches of emulsions, placed in Cryovac bags, and frozen at 10°F. The day before an experiment, the frozen portions were removed from the freezer and allowed to thaw partially. The meat was ground through a 5/8-inch plate and then through a 3/16-inch plate; the fat was ground through a 5/8-inch plate. The weights (kg) of the components of each batch of emulsion were: beef, 6.13; pork, 4.63; fat, 2.86; water-ice, 2.72; salt, 0.36; sugar, 0.27; $NaNO_3$, 0.017; $NaNO_2$, 0.0021; spice mix, 0.072—for a total of 17.01 kg—plus any other additives. The beef and pork were placed in the silent cutter, followed immediately by the water-ice, spices, cure, salt, and sugar. After 3 min of chopping the fat was added. The initial temperature was about 35°F. If reductants were used, they were added at the level of 7/8 oz per 100 lb of meat (7.8 g/17 kg emulsion). GDL was added at the level

and pH and nitric oxide hemochrome content were determined.

An examination of curves representing the formation of nitric oxide hemochrome during this cooking disclosed that they could be classified in three categories (Fig. 1). Color production was fastest in emulsions containing ascorbate, with or without GDL, which had been chopped in air and vacuum-mixed (Curve A). The treatments of intermediate effectiveness included all of the emulsions chopped in nitrogen, regardless either of reductant or whether they were vacuum-mixed; the emulsions prepared without added reductant, or with cysteine, which had been chopped in air and vacuum-mixed; and the emulsions with ascorbate, with or without GDL, which had been chopped in air but not vacuum-mixed (Curve B). The group in which color developed the most slowly included the emulsions either without added reductant, or with cysteine, both of which had been chopped in air and not vacuum-mixed (Curve C). Less than 50% conversion developed in these emulsions on cooking to 160°.

During cooking, the time-temperature relationship applied was designed to approximate that used in commercial smokehouse practice. Under these conditions, Curve B, typical of most of the various treatments, can be taken to represent a normal rate of color production. Color production occurred mainly as the temperature increased from 120° to 140°, with essentially complete color production at 150°F.

The cooking studies show the effect of removing oxygen by chopping under nitrogen, or vacuum mixing. As shown in Fig. 1, applying either of these treatments to the emulsions with no added reductant, or with cysteine, increased the rate of nitric oxide hemochrome formation to that observed in
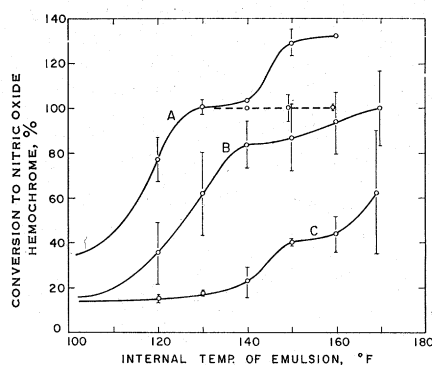

Fig. 1. Conversion of heme pigments to nitric oxide hemochrome on cooking emulsions with treatments as follows:

Curve A, emulsions containing ascorbate, or ascorbate plus glucono-delta-lactone, chopped in air and vacuum mixed (dotted line shows correction for liquid loss).

Curve B, all six emulsions chopped under nitrogen; emulsions with no added reductant, or containing cysteine, that were chopped in air and vacuum mixed; emulsions with ascorbate, or ascorbate plus glucono-delta-lactone that were chopped in air and not vacuum mixed.

Curve C, emulsions with no added reductant, or with cysteine, that were chopped in air and not vacuum mixed.

the emulsions containing ascorbate. The low rate shown by Curve C is typical of emulsions in which the elimination of oxygen proceeded slowly.

Considering the treatments that yielded color most rapidly (Curve A) as a separate category may be questionable. Approximately 35% nitric oxide hemochrome had developed in the emulsions during vacuum mixing, but the rates of color increase in curves A and B are essentially the same. Furthermore, one characteristic of Curve A resulted from an untoward effect of the vacuum mixing: partial destruction of the emulsion. The emulsion contained an apparent 130% of the total heme pigment as nitric oxide hemochrome because of a loss of fat and water from the emulsions at 140-

150°F, concentrating the pigment. As shown by a correction of values in Curve A for liquid losses (dotted line in Fig. 1), the amount of color formation shown by Curves A and B was not significantly different at temperatures above 140°F.

pH changes during cooking. In general, pH values rose from an initial value of 5.45 to a constant value of 5.90 on cooking to 150°F. However, when GDL was used, the pH remained constant during the entire cooking procedure after the previously mentioned decrease of 0.15 pH units. This result is in agreement with the conclusion expressed by Sair (1965) that GDL hydrolyzes slowly after addition to the emulsion. The present results indicate that the rate at which basic groups were exposed as the proteins in the emulsions became denatured, corresponded with the rate at which the lactone hydrolyzed, thus maintaining a constant pH.

Storage. Batches of emulsion without added reductant, or containing either cysteine, erythorbate, ascorbate, erythorbate and GDL, or ascorbate and GDL, were chopped either in air or under nitrogen atmospheres to 62–64°F, and portions were vacuum-mixed for 1 min. Some samples, either with or without vacuum mixing, were cooked immediately, and others were tempered at 80° or 100°F for 2 hr. They were then cooked in baths in which the temperature was gradually raised from 120° to 170°F. Samples were withdrawn at internal temperatures of 130° (45 min), 156° (1.5 hr), and 170°F (2 hr) and chilled. Samples were wrapped either in Saran or in polyethylene, and lots were stored at 35 and 45°F. Nitric oxide hemochrome content and pH were determined after chilling and after various periods of storage.

The results of storage at 45°F are

Table 2. Nitric oxide hemochrome as percent of total heme in emulsions stored at 45°F.

Cooking temp (°F)	Storage time (days)	Packaging film	Experiment no.												
			1		2		3		4		5		6		
			AAN[a]	AAV	ANN	ANV	CAN	CAV	CNN	CNV	EAN	EANG	NAN	AAN	AANG
130	0		46	63	61	....	13	70	25	88	54	54	13	58	52
	6–18	Permeable	20	31	17	....	34	45	40	58	22	57	26	26	64
		Impermeable	73	71	55	....	53	60	59	69	60	63	45	65	64
156	0		66	67	49	57	44	71	73	98	59	61	42	52	76
	6–18	Permeable	55	51	45	46	37	44	44	58	48	46	36	44	50
		Impermeable	61	59	48	47	43	47	47	61	49	50	44	45	49
170	0		74	....	59	....	62	....	67	....	....	....	51	....	....
	6–18	Permeable	71	....	50	....	44	...	53	....	....	....	44	....	....
		Impermeable	70	....	53	....	49	....	59	....	....	....	48	....	....

[a] First letter indicates reductant: A for sodium ascorbate; C for cysteine; E for sodium erythorbate; and N for none added. Second letter indicates atmosphere during chopping: A for air and N for nitrogen. Third letter indicates vacuum mixing: N for none and V for 1 min. Fourth letter, G, indicates that glucono-delta-lactone was added.

Table 3. Summary of t tests based on average nitric oxide hemochrome content during 6th to 18th days of storage at 45°F.

Added reductant	Atmosphere of chopping air/ nitrogen	Vacuum mix none/ 1 min	Tempering none/ 2 hr @ 80°	Cooking 130/156°F		Packaging Perm./Imperm.	
				Permeable	Imperm.	130°	156°
None	....	....	n.s.	< (0.02)	n.s.	< (0.1)	< (0.02)
Cysteine	<[a,b] (0.005)[c]	<[d] (0.005)	n.s.	<[e] (0.05)	>[e] (0.001)	<[e] (0.001)	n.s.
Erythorbate	....	....	n.s.	< (0.001)	> (0.002)	< (0.001)	n.s.
Ascorbate	n.s.	n.s.	n.s.	< (0.001)	> (0.01)	< (0.001)	n.s.
Erythorbate + GDL	....	....	n.s.	> (0.03)	> (0.03)	n.s.	n.s.
Ascorbate + GDL	....	....	n.s.	> (0.005)	> (0.005)	< (0.02)	n.s.

[a] Symbol (<) shows which of two treatments (air/nitrogen) was the more effective.
[b] Irrespective of mixing, cooking temperature, or packaging film.
[c] Levels of significance in brackets; n.s. = not significant.
[d] Irrespective of atmosphere of chop, cooking temperature or packaging film.
[e] Irrespective of atmosphere of chop or cooking temperature.

Table 4. Effect of tempering on percent conversion to nitric oxide hemochrome during processing of the samples prior to storage.

Holding	Cooking (°F)	AAN[a,b]	CAN	CNN	NAN
None	130	46	13	25	13
	156	66	44	73	42
2 hr at 80°	130	77	13	21	9
	156	73	68	65	43
2 hr at 100°	130	78	23	34	....
	156	65	61	....	....

[a] For coding, see footnote a, Table 2.
[b] These were the only groups in which the tempering or lack thereof made a difference in the initial color before storage.

shown in Tables 2 and 3. Results during storage at 35°F were very similar. Selected data on the effect of tempering are given in Table 4. The data in Table 2 are the results of analyses of the 13 emulsions cooked to the three different temperatures—at the time the samples were placed in storage and after the samples had been stored for *ca.* 6–18 days in the two types of wrappers. Inasmuch as statistical analyses showed that the effect of varied tempering was not significant with respect to the color of the stored samples, the data on tempered and untempered samples were averaged. The data in Table 3 are comparisons of average nitric oxide hemochrome content during the 6–18th days of storage based on t-tests (Pearson and Hartley, 1958).

During storage, the nitric oxide hemochrome content of most of the samples tended to approach values that were principally characteristic of the temperature of cooking and the wrapping film. Two phases of the change in color were observed, a first phase characterized by a rapid initial change during the first 4–6 days of storage, and a second phase, lasting up to 18–20 days of storage, during which the color either remained stable, or decreased at a slow and relatively constant rate. If conversion during processing was high (60–70%), the emulsion lost color during the first phase; if the conversion was low (below 40%), nitric oxide hemochrome content increased. The nitric oxide hemochrome content during the second phase was higher in the samples wrapped in Saran (oxygen-impermeable) than in the samples wrapped in polyethylene.

In the samples cooked to 156°F, the change during the initial phase of storage was very small. The average value during the second phase was $47.5 \pm 4.5\%$, and was independent of wrapping film except in the emulsion containing no added reductant. In the samples cooked to 130°F, the changes during the first phase were relatively high. If the samples were wrapped

in Saran, the nitric oxide hemochrome content averaged about 60% and the color was stable, that is, the changes were statistically insignificant during the second phase. The samples cooked to 130° and wrapped in polyethylene had a lower nitric oxide hemochrome content during the second phase than the corresponding Saran-wrapped samples. The color of the samples wrapped in polyethylene was characterized by a slow loss of nitric oxide hemochrome of about 0.5% per day. This rate of change of color was less than the experimental variation, and 3–4 determinations made periodically during the second phase were consequently taken as a reliable estimate of the color concentration during this period.

Effect of chopping under nitrogen and vacuum-mixing. These two treatments are considered together since the end result of each was the removal or reduction of oxygen in the emulsions. In accord with previous observations, of the two emulsions studied with respect to chopping under nitrogen and/ or vacuum mixing, only the emulsions with added cysteine had an improved color as a result of the treatments. In addition to improving the initial color of the cooked samples, the two treatments also improved the color during storage (*cf* Table 3). The treatments, in decreasing order of effectiveness, were: cysteine, chopped under nitrogen and vacuum-mixed; cysteine, chopped in air, and vacuum-mixed and also cysteine chopped under nitrogen, not vacuum-mixed; and cysteine, chopped in air, and not vacuum-mixed.

Tempering. As previously noted, tempering had no significant effect on the color of the stored samples, but in some emulsions there was an effect on the initial color (Table 4). As can be seen, tempering was effective in improving the initial color in the emulsions containing either ascorbate cooked to 130° or cysteine cooked to 156°, but was less effective applied to emulsions containing cysteine cooked to 130°. These low conversions are the result of the lag period previously observed in the holding and cooking experiments. To sum all of these observations in terms of a tempering period, a holding or tempering period was fully effective only if it resulted in completion of those changes, mainly the scavenging of oxygen, which allowed the conversion to nitric oxide hemochrome to proceed. Tempering before cooking was effective only if the time interval encompassed the lag period, but usually was not necessary if the emulsions were cooked to 156°F.

Cooking temperature and wrapping.

As previously mentioned, the effect of varying the temperature of cooking on the level of color maintained during the 6–18th-day period depended on the wrapping used during storage. Cooking at 156° produced a more rapid rate of color production than cooking at 130°, which, coupled with the additional time required to reach the higher temperature, permitted more complete development of color in all emulsions. The effects of the two temperatures of cooking and of the two wrapping films on the nitric oxide hemochrome content during storage have already been described, and, in summary, were marked by the relative stability of the color of the emulsions cooked to 156°, and the large changes occurring in the emulsions cooked to 130°. The latter involved an increase in nitric oxide hemochrome in samples wrapped in Saran, and a decrease in samples wrapped in polyethylene.

These observations are of importance with respect to the cause of the relative stability observed to be characteristic of frankfurters cooked to 156° as compared with products cooked at lower temperatures, for example, 130°F. It could be assumed that the denaturation of the proteins in the emulsion was associated with the observed stability, but no real justification of this view has been given. Part of the difficulty lies in an uncertainty as to just what the cured-meat chromophore is (Tarladgis, 1962). It appears logical to assume that one important difference between cooking to 130° and 156° is the effect of additional heating on the environment of the pigment, which could influence its form. In the samples in this study it did not appear that denaturation of the proteins was complete at 130°, as evidenced by the relatively soft and spongy character of the emulsions. At 156° the emulsions were quite firm and it appeared that the process of denaturation was very largely complete. As for the heat denaturation of the pigment, myoglobin, Fox (unpublished) found that appreciable denaturation of the purified pigment did not occur until 160°F during periods normally used in cooking emulsions. Bernofsky et al. (1959) found that approximately 30% of the heme pigments of beef and pork could be extracted even after 300 minutes of heating. Tests were run in the present study on various emulsions cooked to 130, 156, and 170° to determine the undenatured (water-soluble) heme pigment concentrations. From the optical absorption at 525 mμ, an isosbestic point for the spectra of metmyoglobin and nitric oxide myoglobin, the extract-

able pigments were determined to be 40% at 130°, 15% at 156°, and 8% at 170°F of the total heme pigment in the emulsions.

The behavior of the samples in the present study furnishes a basis for a better understanding of the mechanisms of color fixation. If it may be assumed that cooking to 130° does not denature the endogenous reducing enzyme systems, or at least only reduces their activity, then some residual production of reducing compounds capable of reforming the nitric oxide hemochrome may occur, and, there being no other drain on the reducing systems, a further production of cured color would result. Such a system was presumed to exist in the Saran-wrapped samples of emulsions cooked to 130°F. If free transport of oxygen into the system were allowed, as in the polyethylene-wrapped samples (130° cook), the action of the electron-transport mechanisms endogenous to the meats would result in a depletion of available reductants through oxidation, and eventually result in color loss. On the other hand, if all of the enzymatic reduction-oxidation processes were completely stopped by denaturation of the enzymes involved, there would be neither production of additional reducing substances nor depletion of the reductants left at the end of the cooking procedure. An equilibrium would then exist between the nitric oxide heme complex and its oxidized dissociated form which would be characteristic of whatever reduction-oxidation systems, enzymatic or non-enzymatic, remained in the emulsions. From these studies it would appear that the existence of such an equilibrium may explain the fact that the pigment composition was approximately 47% nitric oxide hemochrome and 53% hemins in emulsions cooked to 156°F, and 61% nitric oxide hemochrome and 39% hemins in the emulsions cooked to 130°F.

Comparison of cysteine and ascorbate. It has been previously concluded that cysteine does not react with oxygen as fast as does ascorbate. This slower reaction may account for the effect of cysteine on the color of the emulsions during the second phase of storage. The results in Table 2 show that cysteine was the more effective in maintaining color during storage of the samples cooked to 130°F and wrapped in polyethylene. It can be concluded that these emulsions had a higher residual reducing capacity, because of a higher concentration of residual reductant.

The results of this study do not indicate much difference between ery-

thorbate and ascorbate in capability for formation or for stabilization of cured meat pigment. Although no analysis was made of the residual reductants in any of the emulsions, it can nevertheless be observed that even the color in the emulsions with no added reductants, if wrapped in impermeable films, was stable for periods up to 21 days. Consequently, lack of residual reductants does not appear to have been critical in the emulsions, provided a satisfactory color was developed on cooking and samples were wrapped in impermeable film. According to a recent survey by *Meat* magazine (1964), 82.5% of all processors wanted a color stability ranging from 5 to 15 days. All of the samples of this study well exceeded the maximum period if wrapped impermeably. The values for the percent of total heme as nitric oxide hemochrome appear to be low, 47–60%, but, in fact, the colors were comparable to those of commercial samples.

Use of glucono-delta-lactone, by the methods of cooking the samples employed in this study, did not produce any appreciable additional initial color. However, the samples with GDL, cooked to 130°, developed a higher than average cured meat color upon storage, regardless of whether they were wrapped in polyethylene or Saran. These samples had a lower initial pH which persisted during storage, but the effect of pH on the reaction is in question. Grau and Böhm (1955), studying the effect of mineral acids on the color of ham slices, found not only that color was stabilized, but also that regeneration of color on the cut, faded surfaces could result, presumably due to lowering the pH. The increase in the rate of production of nitric oxide heme pigments at low pH values has been observed many times (Brooks, 1937; Watts and Lehmann, 1952a,b; Fox and Thomson, 1963), but it has also been observed that at least one of the reactions of nitrite which causes decomposition of the heme pigments increases at lower pH values (Fox and Thomson, 1964). Bailey et al. (1964), studying the effect of light on the stability of the nitric oxide heme pigments of ham, found that stability was greater in the pH range 6.2–6.4 than in the pH range 5.7–6.0, but this stability may pertain only to dissociation by light, not necessarily to the subsequent oxidation of the dissociated compounds. The dependence of the stability of the color of cured meats has not yet been thoroughly studied over a wide enough range of pH values. However, while no final conclusion can

be drawn for the observed effect of GDL in the emulsions, it appears significant that this effect was pronounced in emulsions cooked only to 130°F, in which an enzymatic reducing system was evidently active.

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